

INFLUENCE OF ASCORBIC ACID AND OF THIAMINE ON PHYSIOLOGICAL RESPONSES OF GUINEA PIGS TO HIGH AMBIENT TEMPERATURE^{1, 2}

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ABSTRACT

The effect of ascorbic acid and thiamine supplementation on response to chronic heat stress was measured in male guinea pigs. Chronic heat stress was associated with decreased food intake, weight loss, and decreased oxygen consumption. Colonic temperatures of heat-stressed guinea pigs remained above normal throughout the stress period. Total leukocyte and hemoglobin levels were significantly lower and eosinophil levels significantly higher in heat-stressed animals. Heart rates were not significantly different during early anesthesia. When body temperature fell to 36°C, heart rates were significantly less in heat-stressed animals. Necropsy indicated decreased liver weight to body weight ratios, decreased percentage of dry weight in adrenals, and increased percentage of dry weight in the livers of animals in the heat. No effect of vitamin supplementation on the physiological changes resulting from heat stress were found. Comparison with the effects of cold stress as reported in the literature indicates that heat and cold are dissimilar in their physiological effect on the guinea pig and that the response to vitamin supplementation is also dissimilar.

INTRODUCTION

The concept that a number of different stress factors could result in the same responses in an organism was introduced by Selye (1936). Among the factors which might elicit the "General Adaptation Syndrome" which he described, Selye suggested exposure to extremes of temperature. Heilbrunn (1952), in accord with this view, states, "Both heat and cold act in similar ways on the protoplasm; when they act as stimulating agents, both cold and heat appear to release calcium into the cell interior."

It seemed reasonable to suggest, therefore, that exposure to excessive heat or excessive cold might result in many of the same reactions in the test subjects, and that diet changes might have the same effects at the two temperature extremes. Therefore, it was postulated that temperature stress, whether heat or cold, is a generalized stimulus to an organism and that temperature stress evokes generalized adaptive responses having survival value. Further, it was postulated that since ascorbic acid is known to modify these adaptive responses in the cold, and since a synergism of action has been observed between ascorbic acid and thiamine (Reid, 1954), these vitamins would also modify adaptive responses in the heat.

MATERIAL AND METHODS

Twenty mongrel, inbred, male guinea pigs, weighing 325-425 gm., were housed in individual cages. Ten animals were kept at an ambient temperature of $22.0 \pm 0.5^\circ\text{C}$ in a constant temperature chamber, while another 10 were kept at $36.0 \pm 0.5^\circ\text{C}$. Relative humidity was controlled in the chamber at less than 50 percent. Dugal et al. (1945) used survival and growth as their criteria of acclimation, but

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in the present study, because of the design of the experiment, it was necessary that all animals survive. Preliminary tests indicated that the 36°C temperature was the highest that was compatible with survival of the animals for the necessary period. Since all the animals did survive, growth became the major criterion of adaptation. It was assumed that any animal in the heat that regained its initial weight or continued to gain weight was acclimated. All animals were acclimated, according to this criterion, at the end of 14 days.

The animals received lettuce, and water *ad libitum*, and a basic ration of a commercial pelleted guinea-pig food.

The 10 guinea pigs used at each temperature were divided into 5 groups of 2 animals each. One group at each temperature served as a control. The other 4 groups received added vitamins dissolved in distilled water. The diets offered to the animals differed only in the amount of ascorbic acid and thiamine (table 1). Vitamins were given orally to each animal daily with a medicine dropper.

TABLE 1
Vitamin supplement solutions administered to four groups of guinea pigs

Diet	Thiamine Hydrochloride Mg./400-Gm. Body Wt.	Ascorbic Acid Mg./400-Gm. Body Wt.	Distilled Water Mg./400-Gm. Body Wt.
A	6	40	.75
B	6	400	1.25
C	60	40	1.5
D	60	400	1.5

A group of physiological variables, reported in the literature to be influenced by either extreme of ambient temperature in one or more homeotherms, was selected as indicators of response to the treatment used. The literature dealing with the effects of high environmental temperature on physiological responses is limited; therefore, since many physiological variables are influenced by heat as well as cold, those which were known to be influenced by cold were accepted as possible indicators for heat responses.

Animals were weighed and colonic temperatures were taken each morning of the exposure period. Beginning on the fifteenth day of exposure, the following final battery of tests was carried out:

(1) Differential leukocyte counts on peripheral blood obtained from an ear vein, and stained with Jenner-Giemsa stain.

(2) Total leukocyte and erythrocyte counts on peripheral blood obtained from an ear vein, following the usual procedure in clinical practice (Gradwhol, 1956).

(3) Oxygen consumption measured in a Minute Oxygen Uptake Spirometer for one hour at the environmental temperature at which the animal had been maintained. The instrument automatically recorded the volume of oxygen used every minute. Preliminary tests had indicated that there was no significant difference between oxygen consumption measurements for 1-, 2-, or 3-hour intervals.

(4) Heart rate monitored by a direct-writing electrocardiograph on the animal anesthetized with sodium pentobarbital. Recordings were made in the constant temperature chamber at the appropriate temperatures. The first measurements were made as soon as there was sufficient anesthetic effect to prevent discomfort when attaching the animal to the machine and were repeated when the animal's colonic temperature had fallen to 36°C.

(5) Hemoglobin measurement on cardiac blood taken immediately following the electrocardiogram and while the animal was still anesthetized. Hemoglobin

level was obtained by the method of Wong (1928), modified by Andes and Northup (1938-1939).

(6) Necropsy of control animals at 22°C and all animals at 36°C. Animals were killed with ether and dissected immediately. Heart, lung, liver, kidneys, adrenals, and spleen were weighed. Samples were also dried and ashed.

Results obtained from the above tests were subjected to analysis of variance. Where this indicated an effect of diet, Duncan's multiple range test (Freund et al., 1960) was used.

RESULTS

Food Intake and Weight Gain

Animals in the heat, as a group, gained less weight than did those at 22°C. Figure 1 shows that most animals in the heat lost weight when first subjected to the heat, while those at room temperature gained weight when put into the constant temperature chamber. There was no significant difference in weight change with variation in the vitamin level. Guinea pigs in the heat ate significantly less ($P=.001$) than did animals at room temperature. Pellet intake was not affected by the vitamin supplementation (table 2).

TABLE 2
Food intake, weight changes, and oxygen consumption in twenty guinea pigs at two ambient temperatures with various levels of vitamin supplementation

Diet	Pellet Consumption Percent of Total Ration Offered		Weight Change Percent of Initial Weight		Oxygen Consumption Liters per Kg Body Weight per Day	
	22°	36°	22°	36°	22°	36°
Control	76	66	14.18	.25	32.52	17.64
	93	87	22.73	24.38	22.59	23.17
Diet A	95	66	25.42	2.48	29.20	20.90
	81	68	19.95	8.05	26.25	24.61
Diet B	96	30	20.69	5.72	30.96	21.03
	94	59	20.94	3.83	26.77	19.49
Diet C	88	57	17.33	-1.74	28.17	22.31
	82	82	18.51	15.16	26.90	19.73
Diet D	100	60	30.21	7.71	27.01	18.66
	100	59	17.50	5.72	26.32	17.90

Colonic Temperature

There was a higher colonic temperature in guinea pigs held in the heat. Their mean colonic temperature on the fifteenth day of exposure was 40.7°C, compared to 39.1°C in those at room temperature. Body temperature did not vary with diet.

Mean daily colonic temperatures of guinea pigs at each ambient temperature are shown in figure 2. It is apparent that the temperatures of guinea pigs subjected to heat increased sharply the first day and then dropped slightly during the following two weeks. They did not, however, return to their original level. It is also clear that there is great variability among animals in the heat.

Oxygen Consumption

Oxygen consumption values were lower in animals at 36°C ($P=.001$) than in those at 22°C (table 2). The various vitamin treatments had no detectable effects.

Hematology

Total leukocyte measurements showed significantly lower values in animals at the higher ambient temperature, but vitamin supplementation was not related to any significant difference in leukocyte count (table 3).

Eosinophil count was significantly higher ($P=.01$) in animals at 36°C, but did not vary with the vitamin intake. Proportions of other cells in the differential count were unrelated to temperature or diet (table 3). Absolute numbers of

TABLE 3
Hematologic values of twenty guinea pigs at two ambient temperatures and on various diets

Diet	Total Leuko-cytes Cells/ Mm. ³ Blood	Total Erythro-cytes Cells 10 ⁶ /Mm. ³ Peripheral Blood	Hemo Globin Gm./ 100 Ml. Blood	Differential Leukocyte Count					Cells with Kurloff Bodies %
				Lympho-cytes %	Neutro-phils %	Baso-phils %	Eosin-ophils %	Mono-cytes %	
22°C Ambient Temperature									
Controls	6762	5.58	10.80	57.5	38.0	1.0	0.5	3.0	0
	7798	5.77	12.12	47.5	50.5	0	0	1.0	1.0
Diet A	11687	7.14	10.15	68.6	29.6	0.2	0.2	1.2	0.2
	7093	6.71	11.19	49.0	48.0	0.5	0.5	2.0	0
Diet B	6952	6.00	10.88	62.5	37.0	0	0	0.5	0
	12864	5.67	10.47	50.8	47.7	0.3	0.5	0.7	0
Diet C	6791	5.39	12.50	71.0	27.0	0	0	1.5	0.5
	8303	7.17	11.83	71.0	28.25	0	0	0.25	0.5
Diet D	8629	5.83	11.07	67.0	31.67	0	0.33	1.0	0
	10619	5.91	11.33	66.7	32.3	0.33	0.33	0.33	0
36°C Ambient Temperature									
Controls	4508	6.88	9.67	61.5	32.0	0	3.5	3.0	0.5
	5194	5.79	11.73	58.5	38.0	0	2.5	0.5	0.5
Diet A	4226	6.03	10.76	49.0	49.0	0	0.5	1.5	0
	4675	7.28	10.66	59.5	36.0	0	1.5	3.0	0
Diet B	3190	6.93	10.33	54.5	39.0	0	4.0	2.5	0
	2898	6.33	10.60	64.0	34.0	0.5	1.0	0.5	0
Diet C	4830	5.73	10.21	34.0	64.0	0.5	1.0	0.5	0
	6871	6.15	11.31	70.0	29.33	0	0	0.67	0
Diet D	2474	5.88	10.15	60.0	38.0	0	0	2.0	0
	2599	6.82	10.36	71.75	26.0	0	1.0	1.0	0.25

lymphocytes and neutrophils were calculated. Analysis of variance of the results indicated a significant decrease in both types of cells in the heated animals, showing lymphopenia ($P=.001$) and neutropenia ($P=.01$).

Analysis of hemoglobin levels (table 3) indicated a significantly lower level in animals at 36°C ambient temperature, but no significant effect of vitamin supplementation ($P=.05$).

Erythrocyte values were unaffected by the ambient temperatures or diets used.

Heart Rate

Heart rates measured when the anesthetic first became effective showed no significant difference with ambient temperature or diet (table 4). When the colonic temperature fell to 36°C, animals from the higher ambient temperature showed a significantly lower heart rate ($P=.001$) than those kept at 22°C.

In animals from the heat, the mean difference between the body temperatures

during the first electrocardiogram and that taken at 36°C was 4.9C°, while in the animals at 22°C, the mean colonic temperature drop was 2.6C°. When change in rate per degree Celsius was calculated, a greater change per degree was shown in heat-exposed animals than in those at 22°C. There was no difference related to change in diet.

TABLE 4
Heart rates and colonic temperatures of twenty guinea pigs at two ambient temperatures and on various diets

	Measurements at First Anesthesia		Measurements at 36°C Colonic Temperature
	Colonic Temperature °C	Heart Rate Beats/Min.	Beats/Min.
22°C Ambient Temperature			
Controls	39.0	333	331
	38.7	316	276
Diet A	38.0	368	333
	38.4	318	324
Diet B	39.0	390	355
	38.7	347	344
Diet C	39.0	302	300
	39.0	342	260
Diet D	38.5	390	345
	38.0	356	329
36° C Ambient Temperature			
Controls	41.1	298	214
	40.8	340	185
Diet A	41.0	345	247
	41.5	333	255
Diet B	41.2	358	235
	41.0	312	276
Diet C	40.8	347	234
	40.6	338	281
Diet D	40.5	285	217
	40.4	338	184

When each temperature group was considered separately, it was found that heart rates at 36°C body temperature were significantly lower than rates shown in the first record ($P=.025$ in room temperature animals; $P=.001$ in animals in the heat). In animals kept at room temperature, there was also a significant difference ($P=.01$) among animals on the various diets. Duncan's multiple range test (Freud et al., 1960) showed (1) heart rates of animals on control diets and diets A and C were not significantly different, (2) heart rates of animals on diets B and D were not significantly different from each other, but were significantly higher than those of animals on control diets and diet C, (3) heart rates of animals on diet A were not significantly different from any of the others.

Necropsy

Comparison of organ weight-body weight ratios of control animals at the two ambient temperatures (table 5) showed a significantly lower ratio for the liver of

TABLE 5
*Percentage of organ weight in body weight of twelve guinea pigs
 (400 to 600 grams body weight)*

Organ	22°C Ambient Temperature	36°C Ambient Temperatures				
		Control Diets	Vitamin Supplements			
			Control Diets	Diet A	Diet B	Diet C
Heart	0.316	0.338	0.386	0.307	0.303	0.291
	0.324	0.309	0.310	0.418	0.329	0.335
Lungs	0.785	0.775	0.574	0.707	0.616	0.660
	0.534	0.679	0.650	0.731	0.736	0.669
Liver	5.042	4.300	4.040	4.764	4.530	4.161
	5.229	3.807	4.140	4.092	3.766	4.184
Spleen	0.219	0.255	0.166	0.127	0.121	0.118
	0.124	0.097	0.138	0.147	0.111	0.138
Kidneys	1.045	1.100	1.027	0.974	0.814	0.906
	0.935	0.782	0.770	0.896	0.920	0.836
Adrenals	0.053	0.047	0.035	0.046	0.034	0.039
	0.056	0.049	0.035	0.040	0.040	0.041

heated animals ($P = .05$). In the animals held at 36°C, there was a significant difference ($P = .05$) in adrenal weight as a result of diet. Adrenals of all animals receiving vitamins were lighter than in controls; however, the adrenals of animals on diet B were not significantly lighter.

Percentages of dry weights (table 6) were significantly lower ($P = .025$) in the adrenals and significantly higher ($P = .05$) in the liver of heated control animals when compared to room temperature controls. Vitamin supplementation was not associated with any change in dry weight. Ash weight percentages did not vary with either temperature or vitamin intake for any of the organs studied.

TABLE 6
Percentage of dry weight in organs of twelve guinea pigs

Organ	36°C Ambient Temperatures					
	22°C Ambient Temperature Control Diets	Control Diets	Vitamin Supplements			
			Diet A	Diet B	Diet C	Diet D
Heart	19.34	19.49	16.79	19.82	19.33	19.84
	20.13	18.73	20.12	20.00	20.00	18.86
Lungs	19.09	17.85	19.39	19.56	19.14	18.67
	20.48	17.25	13.95	18.01	15.06	18.30
Liver	23.23	24.55	24.51	22.42	26.05	24.96
	23.07	25.25	24.51	22.69	24.34	26.56
Spleen	20.63	21.88	21.12	20.71	21.75	22.13
	21.58	21.23	21.18	19.97	22.28	21.05
Kidneys	21.44	20.38	20.41	21.19	22.78	21.31
	19.60	21.75	22.40	18.76	20.88	20.34
Adrenals	32.04	29.51	31.83	36.81	29.02	29.10
	32.83	29.82	30.91	30.64	29.79	30.60

DISCUSSION

Effects of Prolonged Heat Exposure

Weight gain.—The decreased food intake observed in these animals in the heat is in accord with observations by others (Brobeck, 1948; Young and Cook, 1955). In the present study, the lessened food intake was partly offset by a decrease in metabolic rate as indicated by the decrease in oxygen consumption in the heat; however, it appears by inspection of weight gains that the lessened rate of metabolism did not completely compensate for the decrease in food intake.

Using the values in table 2 as a basis for calculation, table 7 was derived,

TABLE 7
*Differences in feed consumption, oxygen consumption and weight gain
in guinea pigs at two ambient temperatures*

Ambient Temp. °C	No. of Animals	Mean Pellet Consumption % Total Ration Offered	Mean O ₂ Consumption 1/Kg. Body Weight	Mean Wt. Gain % Initial Value	Per Cent of Room Temperature Values		
					Pellet Intake	O ₂ Consump.	Wt. Gain
22	10	90.5 ±7.8	27.6 ±2.61	20.75	100	100	100
36	10	63.4 ±14.7	20.5 ±3.83	7.16	70.1	74.5	34.5

showing that pellet intake of animals in the heat was 70 per cent of the intake of animals at 22°C, or 30 per cent less, while oxygen consumption was 25.5 per cent less. During their period in the heat, mean weight gain of the animals was 7.2 per cent of the initial weight, while for those held at room temperature, mean weight gain was 20.8 per cent of the mean initial weight. It is obvious from the above figures that animals in the heat gained weight at a rate that was only 34.5 per cent of the rate found in room temperature animals. Therefore, one effect of prolonged heat exposure in these guinea pigs was decreased food intake and slowing of the growth rate, which occurred despite some compensating decrease in energy expenditure.

Body temperature.—Studies on exposure to heat in human beings have indicated that one effect is an initial rise in body temperature which tends to return to normal as the process of acclimation proceeds (Bean and Eichna, 1943; Bass et al., 1955; Robinson et al., 1943; Machle, 1944).

In the guinea pigs in the present study, colonic temperatures increased markedly the first day in the heat and then declined slowly. But colonic temperatures did not ever return to normal during the prolonged exposure to the heat, nor did the animals show as great a drop in temperature during this period as Robinson et al. (1943) reported in their human subjects.

According to Prosser and Brown (1961), rodents are poor heat regulators. They may wet their fur with available water or saliva and thus may increase cooling by evaporation. In the guinea pigs in this study, heat regulation by wetting the fur was never observed. In addition, according to Sullivan and Mullen (1954), the thick skin and heavy coat of the guinea pig act as good insulators and also has a low conductance value (Herrington, 1940). Figure 2 shows that there is greater variation in colonic temperature among the animals at the higher ambient temperature, indicating greater variation in ability to regulate body temperature in the heat.

Oxygen consumption.—The zone of thermal neutrality in guinea pigs has been reported to be in the range of 30.0 to 30.9°C (Herrington, 1940). In the present study, the two ambient temperatures used were approximately 8°C below and 5°C above this temperature range, respectively. As a result, it is assumed that the oxygen consumption values obtained do not represent the minimum.

In this study, there was less oxygen consumed per kilogram of body weight at the higher ambient temperature. However, since the zone of thermal neutrality lies between the two ambient temperatures used, it cannot be assumed that the oxygen consumption decreases in linear fashion with an increase in ambient temperature. It seems likely that oxygen consumption would have been greater in the animals at 36°C than in control animals, if the control temperature used had been in the zone of thermal neutrality.

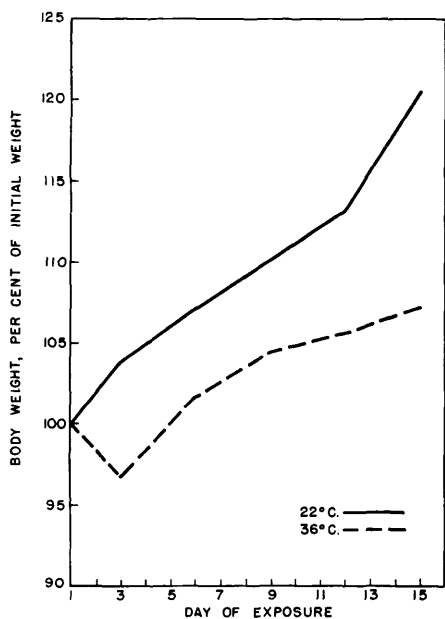


FIG. 1

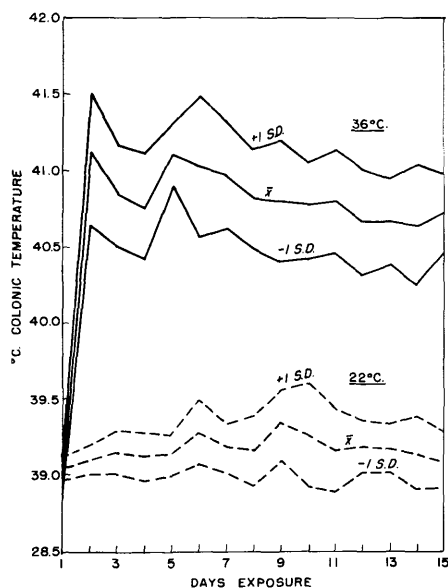


FIG. 2

FIGURE 1. Mean Body Weights as Percentage of Initial Weights in Guinea Pigs Exposed to Two Ambient Temperatures.

FIGURE 2. Mean Daily Colonic Temperatures of Guinea Pigs at Two Ambient Temperatures.

Heart rate.—It is generally accepted that the heart rate increases with an increase in body temperature. It would be valid to assume, then, that the increased body temperatures of animals at 36°C ambient temperature in this study would have been accompanied by an increased heart rate. However, as Selye (1950) has remarked, there are few or no studies on cardiovascular function during the resistance phase of the “general adaptation syndrome.”

In this study, results show a significant decrease in heart rate in any single animal as the body temperature dropped. This change occurred over a short period of time, usually less than an hour. These results are in agreement with studies of the effect of temperature change over short periods.

However, as Herrington (1949) states, “Varying degrees of acclimatization and length of exposure make a simple connection of rate and temperature unreliable.” Cardiovascular changes are believed to be the major mechanism of acclimation during longer periods of heat exposure. For example, there have been found to

be progressively smaller increases in pulse rates in man during a prolonged heat exposure (Bean and Eichna, 1943; Robinson et al., 1943).

Evidence of a change in the heart rate-temperature relationship following acclimation was indicated in this study by two facts. First, there was no significant difference in the first heart-rate records between animals at the two ambient temperatures, even though their body temperatures differed markedly. Second, at a colonic temperature of 36°C, the heart rates of animals that had been held in the heat were significantly lower than heart rates of animals held at room temperature. It is suggested that the effect of acclimation may account for these results.

Following the vasodilation which occurs on exposure to heat, there has been shown to be an increase in blood volume in man (Bazett et al., 1940; Conley and Nickerson, 1945). As a result of this increased blood volume, a rapid heart rate would no longer be needed to maintain the blood pressure. It might be assumed, then, that heart rate would tend to decrease even though the body temperature remained high.

The more rapid decline in heart rate in animals from the heat as the body temperature fell might be explained on the basis of a fluid shift. This would also account for the lower heart rate found in animals from the heat when the colonic temperatures of both groups had fallen to 36°C. When the sodium pentobarbital was given, there was a fall in body temperature. Barbitol anesthetics have been found to suppress shivering more than they suppress vasomotor reflexes (Hemingway, 1941). Therefore, vasoconstriction could cause a rise in blood pressure, and the blood pressure would be greater in animals in the heat because of the larger blood volume. As a result, there would be a greater drop in heart rate.

Another possibility may be that the animals had adjusted so that they now had a normal heart rate at the higher temperature, as part of the acclimation process.

It must be pointed out that the effect of the anesthetic is an unknown factor here. Richards and Taylor (1956) state that environmental and body temperatures are complicating factors in determining the action of barbiturates, but they conclude that no general agreement exists on the effect of temperature on drug action. A further complication is the change in dosage with temperature. This was required since it appeared that the drug increased in its action at the higher temperature. It seems probable that the decrease in dosage would largely compensate for the apparent increase in drug action at the higher temperature, but this would be only approximate.

Erythrocyte level.—Bazett et al. (1940), Conley and Nickerson (1945), and Spealman et al. (1947) report that exposure to high temperatures results in an increase in blood volume. The assumption might be made that hemodilution would result in decreased erythrocyte level. The absence of effect of ambient heat on erythrocyte levels in the animals in this study seems to indicate that, if a fluid shift had occurred, it had either reversed itself or been compensated for before the end of the 2-week exposure period.

In man, the blood is believed to be diluted primarily by fluid from skin, muscle, and liver tissues. Liver weights of guinea pigs exposed to heat in this study were significantly less, and dry weights significantly more, than in room-temperature animals. These facts would lend support to the possibility that a fluid shift had occurred.

Secondly, it is well known (Wintrobe, 1961) that hemoglobin formation lags behind red-cell formation during regenerative processes. If erythrocytes were formed at a rapid rate and hemoglobin lagged somewhat behind, this might account for the lower hemoglobin levels found in the heat-exposed guinea pigs in this study. It seems possible then, that a fluid shift occurred, with increased erythropoiesis, followed by hemoglobin regeneration.

The lack of change in erythrocyte level is in agreement with Dalton and Selye (1939), who noted an increase in the blood during the shock phase of the "alarm reaction," followed by a return to normal or below, presumably because of blood dilution.

Hemoglobin levels.—The decrease in hemoglobin levels found in the heat-exposed animals in this study may be a consequence of the fluid shift, as already mentioned above. Another possibility which cannot be overlooked, however, is that the decrease might be related to the decrease in total food intake. Since many nutrients are involved in hemoglobin formation, one of these nutrients might have become deficient when the pellet intake decreased.

Leukocyte levels and differential counts.—A survey of the literature suggests several mechanisms by which leukopenia could have been produced. First, Ershoff and Gaines (1953) refer to reports that leukocyte counts in peripheral blood are greater than in cardiac blood. Quimby and Goff (1952) postulate that there is a "damming up" of leukocytes in peripheral areas of the vascular system as a result of normal resistance to blood flow in arterioles and capillaries. Ershoff and Gaines (1953) suggest that the vasodilation in heat-stressed animals and the increased circulatory rate tend to reduce the peripheral vascular resistance, leading to a more equal distribution of leukocytes and a subsequent decrease in leukocytes in peripheral blood. While the work of these authors was carried out on rats for short heating periods, it seems possible that the effect described could be continued for longer periods of time as long as the heat stress continued, as was done in this study.

Another possibility is the fluid shift mentioned previously. The increase in total blood volume might have a diluting effect on leukocyte concentration in the blood.

A third possibility is suggested by the work of Doan (1938), who found leukopenia in rabbits as long as fever therapy continued as a result of changes in bone marrow and lymph nodes. The leukopenia observed could, then, be the result of increased cell destruction and decreased ability to form new cells in the heat.

These same mechanisms, either singly or in combination, could also explain the lymphopenia and neutropenia found in the present study.

Necropsy findings.—A possible reason for the significantly smaller livers in proportion to body weight found in heated animals in this study and their larger proportion of dry weight has been considered above. The fact that there was no significant difference in organ-weight to body-weight ratios for the other organs considered indicates that the slower growth of heat-exposed animals did not have a greater effect on any one organ. However, in view of published work on the relationship of stress and the adrenal gland, the lack of change in adrenal size in the animals in the heat in this study deserves discussion.

Selye (1950) has stated that the adrenal cortex reacts in approximately the same manner in response to a variety of non-specific stressors. Among the changes which he describes are hypertrophy of adrenal cortical cells. If it is assumed that heat is a non-specific stressor, then it would follow that the adrenal gland should be hypertrophied in the heat-stressed guinea pigs in the present study.

The lack of adrenal gland enlargement in the animals in this study is in agreement with results obtained in rats by Hale et al. (1959) and Herrington and Nelbach (1942). The following factors seem to be possible explanations for the results obtained:

- (1) Adrenal hypertrophy may have occurred early in the heat exposure and be followed by a return to normal size with acclimation.
- (2) The effect of heat on the adrenal glands might be considered a specific stress which does not elicit the typical reaction of the "General Adaptation Syndrome" elicited by non-specific stressors. The apparent lack of adrenal hypertrophy might, then, be considered a specific effect of the heat rather than

a non-specific response. Heat would not, then, be considered a non-specific stressor.

(3) Adrenal weight may be decreased as a result of decreased food intake in heat-exposed animals.

The significantly smaller dry weight of the adrenals from heat-stressed animals in the present study is apparently a further indication of a decrease in adrenal tissue. In the absence of histological studies and lipid analysis, its significance cannot be elaborated.

An indication of the level of adrenal cortical activity is offered by the eosinophil level in the blood. It is commonly accepted that increased secretion of adreno-cortical hormones results in a decreased eosinophil level in the blood. The higher eosinophil level in the heat-exposed guinea pigs indicates a decrease in adreno-cortical activity. Since the tendency to lower adrenal weights might also indicate decreased hormone secretion, these two findings are in apparent agreement. However, it is important to note that adrenal size and activity do not necessarily vary directly (Heroux and Schonbaum, 1959).

Effects of Vitamin Supplementation

Heart rate in room temperature animals.—Animals at room temperature on diets B and D had a significantly higher heart rate than those on the other diets or the controls. Guinea pigs treated with ascorbic acid have been found (Green and Musulin, 1941) to show increased tolerance to a number of anesthetics. It is suggested that the results obtained in this work might indicate an increased tolerance to sodium pentobarbital anesthesia as a result of the ascorbic acid supplementation. Because pentobarbital has an effect which tends to lower the heart rate, an increased tolerance could be indicated by a faster heart rate.

Since no effect was indicated in heat-exposed animals, it cannot be said that the vitamin supplementations at the levels used had an effect on the response of these animals to heat stress.

Adrenal gland to body weight ratio.—The role of ascorbic acid in the stress-induced adrenal hypertrophy is unknown. In this study, vitamin supplementation apparently had the effect of decreasing relative adrenal size. If it is assumed that the effect is a reduction of a stress-induced hypertrophy, the question arises as to the nature of the stress. The data presented here have shown that the adrenal glands in the animals have not hypertrophied in the heat; therefore, the vitamin supplementation cannot be counteracting a heat effect. It seems possible, however, that the vitamins could be counteracting a stress-induced hypertrophy caused by other stresses, namely, any or all of the experimental procedures to which the animals were subjected prior to necropsy. It seems reasonable to suppose that the total effect of these procedures could result in adrenal hypertrophy in all the animals, which is counteracted in those fed the vitamin supplements. Under these circumstances, then, it cannot be assumed that the vitamin supplementation has had an effect on the animals' adrenal response to the heat stress.

In none of the other variables measured was there any evidence of an effect of the vitamin supplementation. Further, in those variables discussed above, where a difference as a result of diet does exist, there is no evidence that vitamin supplementation had any effect on the physiological changes which occurred as a result of heat stress.

Comparison with the Effects of Cold Acclimation

In view of Selye's theory of the common effect of various stressors, it is interesting to compare the results obtained in the present study with results of cold acclimation reported in the literature. Only the effects of relatively long periods of cold exposure in which there is assumed to be some degree of acclimation will be considered here.

Energy exchange.—In cold-acclimated rats ($0 \pm 4^\circ\text{C}$), there is increased metabolic rate, increased food intake, and maintenance of normal body temperature, but a slower growth rate (Therien, 1949). In contrast, in the heat-acclimated animals in the present study, decreased metabolic rate and decreased food intake were found. The colonic temperature increased on initial heat exposure, but showed only little tendency to return to normal. As in cold-acclimated animals, there was an initial weight loss, with a tendency to regain weight as acclimation progressed, but failure to reach weight gains found in control animals.

It appears, then, that although there is inhibition of growth at both temperature extremes, the reasons for these results are different. In both cases, there is an apparent caloric deficit which results in growth failure. In the cold, the increased food intake does not seem to compensate for the increased metabolic rate; while in the heat, the decreased food intake was not compensated for by the decrease in metabolic rate.

A contrast is also shown in the effect of ascorbic acid. Rats and guinea pigs receiving ascorbic acid supplements lost less weight and gained weight sooner during cold exposure than did the controls (Dugal and Therien, 1949; Therien, 1949). Results of the present study did not show any effect of vitamin supplementation on weight gain in the heat.

Ascorbic acid supplementation was shown (Dugal and Fortier, 1952–1953) to promote the capacity of cold-acclimated monkeys to maintain body temperature, while, in the present study, no effect of vitamins on body temperature was found. A review of the literature failed to reveal any studies of the effect of vitamin intake on the food intake or oxygen consumption in cold-acclimated animals.

Heart rate.—Heroux et al. (1959) compared heart rates in adult rats kept at 30°C and at 6°C for 82 days and found that rates were consistently higher in the 30°C -animals when the rates were measured at a variety of ambient temperatures. These results appear to contrast with ours for the heat-acclimated guinea pigs, whose heart rates were the same as those in the controls, but whose colonic temperatures were higher. Also, our heat-acclimated guinea pigs had lower heart rates than those of the controls when both groups had colonic temperatures of 36°C . It must be pointed out, however, that the bases for measurement are not the same, for Heroux et al. measured heart rates at a variety of ambient temperatures, while the present study related heart rate to colonic temperature. Nevertheless, the report of Heroux et al. (1959) does indicate that the heart rate in rats did not return to normal with acclimation to cold as it did in the heat-acclimated animals in our study.

No reports were found on the effect of vitamin supplementation on heart rate in cold-acclimated animals; therefore, no comparisons can be made.

Erythrocyte and hemoglobin levels.—Stullken and Hiestand (1954) found no significant change in erythrocyte levels after exposing mice to 5°C for 5 days. Heroux (1961) and Sealander (1962), in reviews of the literature, indicated that hemoglobin levels increased in the cold in some animals and did not change in others. These results are, therefore, also at variance with the results from heat-acclimated guinea pigs in the present study.

No literature was found on the effect of vitamin supplementation on these variables in cold-acclimated animals; therefore, no comparison can be made.

Leukocyte levels and differential counts.—The total leukocyte level has been found to be decreased in guinea pigs after a cold exposure of 4 weeks (Therien, 1949). Ascorbic acid (10 milligrams per day) was reported to protect against the leukopenia found in animals receiving only 2 milligrams per day. A similar effect was not found at room temperature.

Leukopenia found in the heat-exposed guinea pigs in the present work was not affected by vitamin supplementation. This may indicate (1) the physiological process which produces the leukopenia is different at the two temperature ex-

tremes, or (2) the process by which the ascorbic acid functions in the cold does not occur in the heat, or both.

Differential leukocyte counts in cold-acclimated men indicated that the initial decreases in lymphocyte and eosinophil levels disappear with acclimation, although the evidence is not unequivocal (Bass et al., 1951). Josey and Lawrence (1932) reported that eosinophil counts in guinea pigs exposed to 2°C for 6 days was slightly, but not significantly, greater than before exposure.

In view of the limited data on the effect of cold acclimation on differential leukocyte count, a meaningful comparison can not be made. In addition, no reports were found in the literature on the effect of vitamin supplementation on differential leukocyte counts in either heat or cold acclimation.

Organ size.—There is general agreement that there is an increase in the weight of the liver, heart, kidneys, and adrenals in cold-acclimated animals (Emery et al., 1940; Herrington and Nelbach, 1942; Heroux and Gridgeman, 1959; Leblond and Dugal, 1943). The evidence of the effect of cold acclimation on the lungs and spleen is conflicting.

The adrenal hypertrophy which occurs in cold-stressed animals has been found to be reduced or prevented by administration of ascorbic acid (Dugal and Therien, 1949; Booker et al., 1955). No reports were found on tests of effects of ascorbic acid on hypertrophy of other organs.

The decreased size of the liver and lack of significant difference in the size of other organs weighed indicates that the effect of heat is not similar to the effect of cold. In addition, Heroux and Schonbaum (1959) state that there is no significant change in water content of the adrenals of cold-acclimated rats. This is in contrast to the heat-exposed guinea pigs, in the present study, whose adrenal glands contained more water than did the adrenals of the control animals.

A comparison of the effect of ascorbic acid on adrenal hypertrophy also fails to show identical action. While ascorbic acid reduces increased adrenal size in cold-stressed animals, there is no evidence from our study that it has an identical action in the heat, since the adrenals did not hypertrophy in the heat. As noted previously, the decrease in adrenal weight observed in this study might have been caused by the action of ascorbic acid on adrenal hypertrophy resulting from other stress factors. In any case, in view of the absence of adrenal hypertrophy in heated animals in the present study, it cannot be assumed that the effect of ascorbic acid is the same in both heat and cold.

Heat and Cold as Non-specific Stressors

On the basis of the evidence available, then, there seems to be little or no reason to conclude that exposure to heat and to cold have the same physiological effect on the guinea pig. Also, there seems to be no reason to suggest that the vitamin supplementation in this study had an effect similar to that found in the cold. There appears to be no evidence that thiamine and ascorbic acid have an effect in the acclimation of guinea pigs to severe heat.

SUMMARY

Male guinea pigs were acclimated for two weeks at ambient temperatures of 22 and 36°C. Animals at each temperature were divided into 5 groups receiving ascorbic acid and thiamine supplements to a normal adequate diet. Effect of the vitamin supplementation on response to heat-stress was observed by measurement of a number of physiological variables. Heat-exposed animals showed decreases in food intake, weight gain, and oxygen consumption. Body temperature was consistently above normal. Blood changes were characterized by increased eosinophil level, decreased absolute lymphocyte and neutrophil levels, and decreased hemoglobin.

Heart rate decreased as body temperature fell under influence of sodium pento-

barbital at both temperatures. The decrease was greater in heat-exposed animals, so heart rate was slower in heat-exposed guinea pigs than in room temperature animals when both were monitored at 36°C colonic temperature. The liver-to-body-weight ratio was decreased in animals held in the heat. Other variables measured showed no change which could be attributed to heat effect.

Adrenal-to-body weight ratios were lower in animals receiving the vitamin supplements, but there was no evidence of heat-induced enlargement of the adrenals. There was no evidence that supplementation with ascorbic acid and thiamine in the quantities used had any effect on the response of guinea pigs to heat exposure at 36°C for 2 weeks. Comparison of the effects of heat exposure in guinea pigs to the effects of cold exposure in various animals reported in the literature revealed dissimilarity in response under the 2 conditions.

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